Arthritis & Rheumatology
DOI 10.1002/art.39332

Ms. ar-15-0542.R1 – Revised version

Development and initial validation of classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis

Authors: Angelo Ravelli[1,2], Francesca Minoia[2], Sergio Davì[2], AnnaCarin Horne[3], Francesca Bovis[2], Angela Pistorio[2], Maurizio Aricò[4], Tadej Avcin[5], Edward M. Behrens[6], Fabrizio De Benedetti[7], Lisa Filipovic[8], Alexei A. Grom[9], Jan-Inge Henter[3], Norman T. Ilowite[9], Michael B. Jordan[8], Raju Khubchandani[10], Toshiyuki Kitoh[11], Kai Lehmberg[12], Dan J. Lovell[8], Paivi Miettunen[13], Kim E. Nichols[14], Seza Ozen[15], Jana Pachlopnik Schmid[16], Athimalaipet V. Ramanan[17], Ricardo Russo[18], Rayfel Schneider[19], Gary Sterba[20], Yosef Uziel[21], Carol Wallace[22], Carine Wouters[23], Nico Wulffraat[24], Erkan Demirkaya[25], Hermine I. Brunner[8], Alberto Martini[1,2], Nicolino Ruperto[2], and Randy Q. Cron[26], on behalf of the Pediatric Rheumatology International Trials Organization, the Childhood Arthritis & Rheumatology Research Alliance, the Pediatric Rheumatology Collaborative Study Group, and the Histiocyte Society.

Authors' affiliations: [1,2]Angelo Ravelli, MD, Alberto Martini, MD: Università degli Studi di Genova and Istituto Giannina Gaslini, Genoa, Italy; [2]Francesca Minoia, MD, Sergio Davì, MD, Francesca Bovis, BiolD, Angela Pistorio, MD, PhD, Nicolino Ruperto MD, MPH: Istituto Giannina Gaslini, Genoa, Italy; [3]AnnaCarin Horne, MD, PhD, Jan-Inge Henter, MD, PhD: Karolinska Institute, Karolinska University Hospital Solna, Stockholm, Sweden; [4]Maurizio Aricò, MD: Azienda Sanitaria Provinciale 7, Ragusa, Italy; [5]Tadej Avcin, MD: University Children's Hospital, Ljubljana, Slovenia; [6]Edward M. Behrens, MD: The Children's Hospital of Philadelphia, Philadelphia, PA, USA; [7]Fabrizio De Benedetti, MD: Ospedale Pediatrico

Received: Apr 02, 2015; Revised: Jul 01, 2015; Accepted: Aug 11, 2015

Bambino Gesù, Rome, Italy; [8]Lisa Filipovich, MD, Alexei A. Grom, MD, Michael B. Jordan, MD, Dan J. Lovell, MD, MPH, Hermine I. Brunner, MD: Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [9]Norman T. Ilowite, MD: Albert Einstein College of Medicine and Children's Hospital at Montefiore, Bronx, New York; [10]Raju Khubchandani, MD: Jaslok Hospital and Research Centre, Mumbai, India; [11]Toshiyuki Kitoh, MD, PhD: Aichi Medical University, Nagakute, Japan; [12]Kai Lehmberg, MD: University Medical Center, Hamburg, Germany; [13]Paivi Miettunen, MD: University of Calgary, Calgary, Alberta, Canada; [14]Kim E. Nichols, MD: St Jude Children's Research Hospital, Memphis, TN, USA; [15]Seza Ozen, MD: Hacettepe University, Ankara, Turkey; [16]Jana Pachlopnick-Schmid, MD, PhD: University Children's Hospital, Zurich, Switzerland; [17]Athimalaipet V. Ramanan, MD: Bristol Royal Hospital for Children, Bristol, UK; [18]Ricardo Russo, MD: Hospital de Pediatria Juan P. Garrahan, Buenos Aires, Argentina; [19]Rayfel Schneider, MBBCh: University of Toronto and Hospital for Sick Children, Toronto, Ontario, Canada; [20]Gary Sterba, MD: Mount Sinai Medical Center, Miami Beach, Florida; [21]Yosef Uziel, MD: Meir Medical Centre, Kfar Saba, Israel; [22]Carol Wallace, MD: Seattle Children's Hospital and University of Washington, Seattle, WA, USA; [23]Carine Wouters, MD, PhD: University Hospital Leuven, Leuven, Belgium; [23]Nico Wulffraat, MD: Wilhelmina Children's Hospital and University Medical Center Utrecht, Uthrecht, The Netherlands; [25]Erkan Demirkaya, MD: Gulhane Military Medical Faculty, Ankara, Turkey; [26] Randy Q. Cron, MD, PhD: University of Alabama at Birmingham, Birmingham, AL, USA.

Corresponding author: Angelo Ravelli, MD, Pediatria II, Istituto G. Gaslini, Largo G. Gaslini 5, 16147 Genoa, Italy. Telephone: +39-010-56362729, fax: +39-010-393324, e-mail: angeloravelli@ospedale-gaslini.ge.it

Key words: Macrophage activation syndrome, systemic juvenile idiopathic arthritis, hemophagocytic lymphohistiocytosis, classification criteria

Short running title: Classification criteria for MAS

Funding source: This work is supported by the American College of Rheumatology (ACR), the European League Against Rheumatism (EULAR), and the Pediatric Rheumatology International Trials Organization.

ABSTRACT

Objective. To develop classification criteria for macrophage activation syndrome (MAS) complicating systemic juvenile idiopathic arthritis (sJIA).

Methods. A multistep process, based on a combination of expert consensus and analysis of real patient data, was conducted. A panel of experts was first asked to classify 428 patient profiles as having or not having MAS, based on clinical and laboratory features at disease onset. The 428 profiles comprised 161 patients with sJIA-associated MAS and 267 patients with a condition potentially confusable with MAS (active sJIA without evidence of MAS, or systemic infection). Next, the ability of candidate criteria to classify individual patients as MAS or non-MAS was assessed by evaluating the agreement between the classification yielded by the criteria and the consensus classification of the experts. The final criteria were selected in a consensus conference.

Results. Experts achieved consensus on the classification of 391/428 (91.4%) patient profiles. A total of 982 candidate criteria were tested *in silico*. The 37 best performing criteria and 8 criteria obtained from the literature were evaluated in the consensus conference. During the conference, 82% consensus among experts was reached on the final MAS classification criteria. In cross-validation analyses, these criteria had sensitivity 0.72-0.73 and specificity 0.97-0.99. Agreement of the criteria with the original diagnosis by the treating physician was high (kappa = 0.72-0.76).

Conclusion. We have developed a set of classification criteria for MAS complicating sJIA and provided preliminary evidence of their construct validity. Prospective validation is required to confirm the high accuracy of the criteria.

INTRODUCTION

Macrophage activation syndrome (MAS) is the term used to describe a potentially life-threatening complication of systemic inflammatory disorders, which occurs most commonly in systemic juvenile idiopathic arthritis (sJIA) and in its adult equivalent, adult-onset Still's disease[1-4]. MAS is characterized by an overwhelming inflammatory reaction due to an uncontrolled and dysfunctional immune response involving the continual activation and expansion of T lymphocytes and macrophages, which results in massive hypersecretion of pro-inflammatory cytokines[5,6].

The clinical picture of MAS is high, nonremitting fever, hepatosplenomegaly, generalized lymphadenopathy, central nervous system (CNS) dysfunction, and hemorrhagic manifestations. Typical laboratory abnormalities include pancytopenia, increased levels of ferritin, liver enzymes, lactate dehydrogenase, triglycerides, D-dimers, and soluble interleukin (IL)-2 receptor α (also known as soluble CD25, sCD25), and decreased fibrinogen levels. A characteristic histopathologic feature of MAS is the accumulation of well-differentiated macrophages exhibiting hemophagocytic activity in bone marrow biopsy specimens or aspirates[7]. Although the estimated prevalence of MAS in sJIA is around 10%, recent reports suggest that subclinical MAS may occur subclinically in as many as 30-40% of sJIA cases[8,9].

MAS can result in progressive multi-organ failure and eventually a fatal outcome. Recent studies indicate a mortality rate of 8%[10,11], making timely diagnosis and prompt initiation of appropriate treatment imperative. However, early recognition of MAS is often challenging, given the lack of a single pathognomonic clinical or laboratory parameter. Furthermore, histopathologic hemophagocytosis may not be present in the initial stages[12,13] and lacks specificity for hemophagocytic syndromes[14]. In addition, the features of MAS may be hard to distinguish from those

conditions that may present with overlapping manifestations, such as flares of sJIA or systemic infections. Recently, a wide disparity in the frequency and severity of the classical clinical and laboratory features across patients has been observed[10,11].

The difficulties in diagnosing sJIA-associated MAS emphasize the need for accurate criteria that aid physicians in identifying MAS in its earliest stages and in distinguishing it from other conditions. The recognition that the syndrome is clinically similar to HLH has led some to recommend use of the HLH-2004 diagnostic guidelines[15]. An alternative approach is based on the application of the preliminary diagnostic guidelines for MAS complicating sJIA[16]. However, although both guidelines have been utilized for detecting MAS in sJIA, each has several limitations[17]. Altogether, the diagnostic difficulties, the recent therapeutic progress[5], and the advances in understanding MAS pathophysiology and underlying genetic defects[5, 18-20] highlight the need for well-established classification criteria.

The primary purpose of the international collaborative project described herein was to develop a set of classification criteria for MAS complicating sJIA, based on a combination of expert consensus, available evidence from the medical literature, and analysis of real patient data.

METHODS

A multistep process strategy was used in developing the classification criteria and included the following phases: 1) Delphi survey among international pediatric rheumatologists aimed to identify MAS features potentially suitable for inclusion in classification criteria[21]); 2) large-scale data collection of patients with sJIA-associated MAS and two potentially confusable conditions; 3) web-based consensus procedures among

experts; 4) selection of candidate criteria through statistical analyses; 5) selection of final classification criteria in a consensus conference; 6) cross-sectional validation of final classification criteria.

Data collection of patients with MAS and confusable conditions. The design, inclusion criteria and data collection procedures of this part of the project have been described in detail previously[10,11,17]. Briefly, international pediatric rheumatologists and pediatric hemato-oncologists were invited to participate in a retrospective cohort study of patients with sJIA-associated MAS and with 2 conditions potentially confusable with MAS, represented by active sJIA not complicated by MAS and systemic infection.

For patients with MAS, collected information included demographic, clinical, laboratory and histopathologic features at 3 time points: 1) last visit before onset of MAS; 2) onset of MAS; 3) full-blown MAS. Because the classification criteria were aimed to identify MAS in its earlier stages, only laboratory data recorded at onset were retained. Data for patients with confusable conditions were also obtained at disease presentation. Except for blood counts and acute phase reactants, values of laboratory parameters were tested using both the original values provided by each local laboratory and the values standardized according to the international standard (SI) unit system based on their normal ranges, as reported[10].

A total of 1,111 patients, 362 with sJIA-associated MAS, 404 with active sJIA without MAS, and 345 with systemic infection were reported by 95 pediatric subspecialists practicing in 33 countries in five continents. The features of MAS and comparison patients have been described elsewhere[10,11,17].

Web-based consensus procedures among experts. At present, there is no single pathognomonic feature for MAS. Furthermore, no validated diagnostic criteria are available. In order to classify patients as having or not having MAS, we, therefore, decided to use expert consensus as the "gold standard". Based on publication records and experience in the care of children with MAS and related disorders, a panel of experts was created, which was composed of 20 pediatric rheumatologists and 8 pediatric hemato-oncologists.

The experts were asked to classify a total of 428 patient profiles as having or not having MAS, based on the clinical and laboratory features recorded at disease onset. The 428 profiles were selected randomly among the 1,111 collected patients and comprised 161 patients with MAS, 140 patients with active sJIA without evidence of MAS, and 127 patients with systemic infection. A selection bias was unlikely, as the characteristics of selected and unselected patients were comparable (data not shown). The experts were purposely kept unaware of the original diagnosis as well as of the overall course of the patient.

Each patient profile included information about the presence or absence of key clinical manifestations, and the value and normal range of laboratory parameters at the respective institutions. Based on these data, all experts were asked to classify each patient as MAS or non-MAS. The minimum level of agreement among experts was set at 80%. If an 80% consensus was not attained, the patient profile was discussed in a further round. Profiles for which consensus was not achieved at the final round were declared non interpretable and discarded from further analyses. Three rounds of voting were used, with comments and voting from participants available, to augment the number of consensus decisions.

All web-based consensus procedures were conducted by the Pediatric Rheumatology International Trials Organization (PRINTO).

Selection of best classification criteria through statistical analyses. All statistical analyses aimed to select the best classification criteria were conducted only on the sample of patients who achieved experts' consensus about the diagnosis of MAS or non-MAS. The cutoff values for laboratory tests were calculated through the receiver operator characteristic (ROC) curve method, by identifying the point on the ROC curve that discriminated best between patients classified as MAS or non-MAS by the experts.

The aim of this exercise was to assess the ability of candidate criteria to classify individual patients as having or not having MAS, and to evaluate the agreement between the classification yielded by the criteria and the consensus classification of experts. Candidate classification criteria were partly derived from the literature and partly generated from the study data.

Literature criteria included: a) the preliminary diagnostic guidelines for MAS complicating sJIA[16]; b) the same guidelines modified with the addition of the item ferritin at various threshold levels (500, 1,000 or 1,500 ng/ml); c) the HLH-2004 diagnostic guidelines[15], adapted by eliminating 3 of the 8 items, because information about presence of hemophagocytosis was not available for both comparison groups, and neither NK-cell activity nor sCD25 levels were determined in all patients. Criteria obtained from the study data were generated in 2 ways: 1) Through the evaluation, by the steering committee of the project, of all combinations of clinical and laboratory variables (see some examples in the online supplementary Table T1) (combination of criteria approach). 2) By assigning a weight to clinical and laboratory variables, on the basis of their association with the diagnosis of MAS made by the experts, through multivariable logistic regression analyses. For each combination of variables that were significantly associated with the diagnosis of MAS in logistic regression models, the rule was to convert the odds ratio of each variable to its percentage value out of a total of 100. Each set of criteria was, then, composed by a group of variables whose sum of weights made up a

total score of 100 (MAS score). The cutoff value in the MAS score that was associated with the higher likelihood of the presence of MAS was obtained by calculating the point on the ROC curve which corresponded to the highest sensitivity and specificity.

A total of 982 candidate classification criteria were tested. For each set of criteria, we calculated the sensitivity (ability of the criteria to identify a patient as having MAS who had been classified as having MAS as per the expert panel), the specificity (ability of the criteria to identify a patient as not having MAS who had been classified as not having MAS by the experts), the positive and negative predictive value, the area under the ROC curve (AUC-ROC), and the kappa value for agreement between the classification yielded by the criteria and the classification made by the experts. Although there was one single model with the highest predictive value (criterion no. 929 in online supplementary Table T1), we generated multiple combinations for comparison because we believed that less predictive models could have more face validity with the experts. Nevertheless, it was established that in order to qualify for inclusion in expert voting procedures at the consensus conference, a set of classification criteria should demonstrate a kappa value ≥ 0.85, a sensitivity ≥ 0.80, a specificity ≥ 0.93, and an AUC-ROC ≥ 0.90. An exception was made for the historical literature criteria, which were retained for further consideration even if they did not meet all statistical requirements.

Selection of the final classification criteria at consensus conference. The International Consensus Conference on MAS Classification Criteria was held in Genoa, Italy, on March 21-22, 2014. The meeting was attended by all 28 experts who participated in web-consensus evaluations and was facilitated by 2 moderators (NR, HB) with expertise in nominal group technique (NGT). The overall goal of the meeting was to decide upon a preliminary set of classification criteria, using a combination of statistical and consensus formation techniques.

A plenary session was first held to illustrate the scope, methodology, and flow of the project, the results of the Delphi survey, the characteristics of patients enrolled in the data-collection, the results of web-based consensus procedures and of statistical analyses of candidate classification criteria, and the methodology of the NGT. Participants were then randomized into two equally sized nominal groups and, using NGT, were asked to decide, independently of each other, upon which of the classification criteria were easiest to use and most credible (face/content validity), ranking the 5 best from 5 (highest face/content validity) to 1 (lowest). All experts were connected by their laptop to a central computer and submitted all their rankings electronically. A series of repeated independent voting sessions were held until the top 3 classification criteria were selected by each voting group. Then, an 80% consensus was attained on the best (final) set of classification criteria in a session with the two tables combined.

Analysis of the association between the variables included in the final classification criteria and the web-based experts' consensus evaluations. The association between the final classification criteria and the web-based evaluations made by the experts was assessed by multiple logistic regression, which used as explanatory variables the individual items included in the final classification criteria and as the dependent outcome the web-based experts' consensus on patients' classification as MAS or non-MAS. The effect was expressed in terms of odds ratios, and 95% confidence intervals were calculated; statistical significance was tested by the likelihood ratio test. The AUC-ROC of the model was used as an indicator of its predictive ability. The purpose of this post-consensus analysis was to evaluate which were the variables that most influenced the experts' decision to classify the patients as having or not having MAS.

Cross-sectional validation of final classification criteria. This analysis was performed by assessing the performance of the criteria, in terms of sensitivity, specificity, negative predictive value, positive predictive value, AUC-ROC, and kappa value, in discriminating patients with MAS from patients with the 2 confusable conditions (combined in a single group), using the original diagnosis made by the caring physician (i.e. the investigator who entered the patient's data in the study website) as the gold standard. This analysis was made on both the entire sample (n = 1,111) and the restricted sample (n = 683) of patients not used for expert evaluations. Only patients who had all items included in the final classification criteria available were used for the analyses.

RESULTS

Results of web-based consensus procedures among experts. After three rounds of web-evaluations, the experts achieved consensus on the classification of 391 (91.4%) of the 428 patient profiles examined (Figure 1). A total of 95 patients were classified as MAS by the experts, 88 of whom had been diagnosed as MAS also by the treating physician; in 3 and 4 patients the original diagnosis had been sJIA without MAS and systemic infection, respectively. A total of 296 patients were classified as non-MAS by the experts, 47 of whom had been diagnosed as MAS by the treating physician. Thirty-seven patient profiles for which an 80% of consensus among experts was not reached were discarded. The comparison of clinical and laboratory features of patients diagnosed as MAS or non-MAS by the experts is shown in Table 1. Overall, patients who had the diagnosis of MAS confirmed by the experts had more severe clinical and laboratory features than those classified as non-MAS.

Selection of candidate clinical and laboratory variables. In univariate analyses, the following 10 variables revealed the greatest ability to discriminate patients with MAS from comparison patients: ferritin, platelet count, aspartate transaminase (AST), lactic dehydrogenase (LDH), triglycerides, alanine transaminase (ALT), fibrinogen, CNS involvement, hepatomegaly, and hemorrhagic manifestations (Table 2). These variables qualified for inclusion in logistic regression analyses aimed to generate candidate classification criteria through the MAS score method. However, the ALT was excluded owing to its close correlation with AST and a slightly lower statistical performance. It was replaced by neutrophil count or albumin, depending on the model.

Selection of candidate classification criteria. Of the 982 sets of criteria tested, 45 were retained for further evaluation in the consensus conference. Of them, 37 (20 generated through the combination of criteria approach and 17 obtained with the MAS score method) were represented by criteria which met the statistical requirements listed in the Methods, and 8 were criteria derived from the literature. The statistical performance of the 20 best candidate criteria is presented in online supplementary Table T1. The definition and statistics for all 982 criteria tested may be provided to the readers upon request to the authors.

Selection of final classification criteria. During the consensus conference, 7 voting sessions were held among the experts until 3 top classification criteria were left (criteria no. 466, 472 and 929 in online supplementary Table T1). Notably, the same criteria were selected

independently by the two tables confirming convergent validity of the selection process After the last voting session, an 82% consensus was reached on the final definition (criteria no. 472 in online supplementary Table T1). A subsequent open discussion led to the decision to include in the final definition the presence of fever as a mandatory criterion and the requirement that the patient should have known or suspected sJIA. The final classification criteria selected in the consensus conference are presented in Table 3.

Association between final classification criteria and web-based experts' consensus evaluations. For this multivariable analysis, complete data were available on 227 patients. The logistic regression model to evaluate which were the variables included in the final classification criteria that most influenced the experts' decision to classify the patients as having or not having MAS is presented in Table 4. All variables were independently correlated with experts' diagnosis. However, the association was much stronger for ferritin and platelet count. The AUC-ROC of the model was 0.99.

Cross-validation of final classification criteria. The evaluation of the ability of the new classification criteria to discriminate MAS from control conditions in the entire patient sample (N = 787/1,111), made using the diagnosis of the treating physician as gold standard, showed a sensitivity of 0.72, a specificity 0.97, a positive predictive value (PPV) of 93.9%, a negative predictive value (NPV) of 84.8%, an AUC of 0.84, and a kappa value for agreement of 0.72 between the diagnosis yielded by the criteria and the diagnosis made by the caring physician. The same analysis made in

the patient sample not included in the expert evaluation (n = 415/683) showed a sensitivity of 0.73, a specificity 0.99, a PPV of 97.4%, a NPV of 85.9%, an AUC of 0.86 and a kappa value of 0.76.

DISCUSSION

Using a consensus formation and statistical approach, we developed a new set of classification criteria for MAS complicating sJIA (Table 3). Because the criteria were established against comparison samples composed of patients with either rheumatologic (active sJIA without evidence of MAS) or non-rheumatologic (systemic infection) conditions, they may encounter the interest of a multidisciplinary range of specialists.

With the exception of fever, the classification criteria do not include clinical manifestations, but are only determined using laboratory variables. This choice is in keeping with the common view that the suspicion of MAS is most commonly raised by the detection of subtle laboratory alterations, whereas clinical symptoms are often similar to confusable conditions or delayed[22]. In a previous analysis of the MAS sample included in the present study, we found that 4 of the 5 laboratory tests which are part of the criteria (ferritin, platelet count, AST, and triglycerides) were among the parameters that showed a percentage change greater than 50% between the last visit before the onset of MAS and the onset of MAS. In the same study, ferritin demonstrated the largest change over time, which underscores its major importance in MAS detection[10] and supports its use as a mandatory criterion. The key value of ferritin in the diagnosis of MAS was corroborated by the observation that it was the parameter that had the greatest influence on the experts' classification of patients as MAS or non-MAS (Table 4).

Although fever did not discriminate between MAS and control illnesses, as it was recorded in all or nearly all patients in each sample, the expert panel considered fever a prerequisite for the presence of MAS. The cardinal diagnostic role of fever is substantiated by the observation that it was the highest-ranked clinical feature identified in the Delphi survey[21]. Unfortunately, we lacked reliable information on the pattern of fever in the three patient groups. However, it is generally accepted that the onset of MAS is heralded by the shift from the high-spiking intermittent pattern typical of active sJIA to a continuous unremitting pattern[3,4,23].

The detection of macrophage hemophagocytosis in bone marrow biopsy specimens or aspirates or reticuloendothelial organ biopsies is another frequent and characteristic feature of MAS. However, because hemophagocytosis is often absent at the early stages of MAS[10,11] and its demonstration requires an invasive procedure, the expert panel deemed it not necessary for the diagnosis of sJIA-associated MAS. Notably, the demonstration of hemophagocytosis is not mandatory in both the HLH-2004 and preliminary MAS diagnostic guidelines[15,16].

Although possibly useful for diagnostic purposes, the classification criteria are primarily intended for use in clinical trials and research studies. Although the criteria revealed high accuracy and face/content validity in consensus and statistical evaluations, it should be taken into account that they were developed using the expert consensus as the gold standard. Note that the experts were asked to differentiate MAS from non-MAS conditions by examining the clinical features and laboratory values recorded at a single point in time (i.e. at disease onset), and were unaware of the patient's clinical course, laboratory values over time, response to treatment, and outcome. This information was, however, available to the treating physician, who made the original diagnosis in the clinical setting. The disparity in the available information may partially explain the high proportion (47/161; 29.2%) of patients diagnosed as MAS by the caring physician who were classified as non-MAS by the experts. It is conceivable

that because the experts were only provided with the information relevant to the development of the criteria, they tended to confirm the diagnosis of MAS only in straightforward and unambiguous cases. Notably, of the 47 patients who had the diagnosis of MAS not confirmed by the experts, 30 (63.8%) also did not meet the final classification criteria, 11 (23.4%) were not assessable due to the lack of the laboratory variables needed to apply the criteria, and only 6 (12.8%) were classified as MAS by the final classification criteria. In addition, only 18 (46.6%) of the 37 patients in whom the expert could not agree on the diagnosis were classified as MAS by the final classification criteria. These findings underscore the consistency of experts' evaluations and support the validity of the final classification criteria.

That the cutoff values for platelet count and fibrinogen included in the criteria are in the normal range of routine laboratory assessments may be regarded as clinically implausible. The same may apply to the cutoffs for aspartate aminotransferase and triglycerides, which are only slightly above the upper normal limits. However, it is widely recognized that children with active sJIA often have increased platelet counts (e.g., above 600-800 x 10^9/l) as well as elevated fibrinogen levels (e.g., above 500-600 mg/dl) as part of the underlying inflammatory process[24,25]. Thus, paradoxically normal values of platelet count or fibrinogen in the setting of otherwise prominent systemic inflammation may raise the suspicion of MAS[10,22]. Because the levels of serum transaminases and triglycerides are generally normal in children with sJIA who do not have other coexistent pathologic conditions (e.g. infectious hepatitis or familial hyperlipidemia), their simple increase above the upper normal limits, combined with the other clinical and lab abnormalities, may be sufficient to herald the occurrence of MAS. This fits with the real world patient data used in these studies to establish cutoff values that distinguish children with JIA with and without MAS.

The analysis of the role of the change in laboratory tests over time in the detection of MAS was a secondary objective of the present project. However, this exercise was only made for descriptive purposes, that is, to identify and rank the laboratory tests whose change was felt by the experts as most important or useful for the early detection of MAS. Because serial values of lab tests were available for patients with MAS, but not for control groups, we could not establish the threshold level of change in each test that had the greatest sensitivity and specificity for the diagnosis of MAS. This precluded the possibility to incorporate the change in laboratory values over time in the classification criteria. Due to space constraints, this part of the study will be reported in a separate manuscript.

Recently, several episodes of MAS have been observed in sJIA patients under treatment with the cytokine blockers canakinumab and tocilizumab in randomized controlled clinical trials and in post-marketing experience[26-28]. Because these agents inhibit the biologic effects of IL-1 and IL-6, respectively, which are among the pro-inflammatory cytokines involved in the physiopathology of MAS[5,29], it is conceivable that MAS episodes developing during treatment with these biologics may lack fever or some of the typical laboratory abnormalities of the syndrome. Clinical symptoms of patients with sJIA-associated MAS receiving tocilizumab were found to be milder than those of patients not receiving this medication[30]. Preliminary analyses in patients who had MAS while receiving tocilizumab or canakinumab have shown that a few cases did not meet the new criteria did so because of the absence of fever or a peak ferritin level < 684 ng/ml [31,32]. However, more data from the real world of clinical practice are needed to establish whether the criteria should be refined to increase their power to pick up the instances of MAS occurring during treatment with IL-1 and IL-6 inhibitors.

Our study should be interpreted in the light of some potential caveats. Patient data were collected through the retrospective review of clinical charts. A retrospective analysis is subject to missing and possibly erroneous data. However, because all patient profiles were reviewed by the experts and the diagnosis of MAS or non-MAS was confirmed only when a high level of consensus was reached, the impact of this potential limitation was likely minimized. Some important diagnostic parameters of MAS, such as sCD25 and sCD163 levels and NK cell activity, could not be assessed owing to their unavailability in all patient samples. However, these biomarkers are not routinely assessed, nor are they timely, in most pediatric rheumatology centers.

In summary, we have developed a set of classification criteria for MAS complicating sJIA and provided preliminary evidence of their validity. These criteria will help standardize the design and conduct of future therapeutic and research studies and contribute to enhancing the knowledge and the awareness of the syndrome. The criteria should undergo a prospective validation process before their widespread use can be recommended.

REFERENCES

1. Prieur AM, Stephan JL. [Macrophage activation syndrome in rheumatic diseases in children]. Rev Rhum Ed Fr 1994;61:447-451

2. Grom AA, Passo M. Macrophage activation syndrome in systemic juvenile rheumatoid arthritis. J Pediatr 1996;129:630-632

3. Sawhney S, Woo P, Murray KJ. Macrophage activation syndrome: a potentially fatal complication of rheumatic disorders. Arch Dis Child 2001; 85:421-426

4. Ravelli A, Martini A. Macrophage activation syndrome. In Pediatric Rheumatology. (T.H. Lehman, R. Cimaz Eds). Elsevier, Amsterdam 2008, pp.55-63.

5. Ravelli A, Grom AA, Behrens EM, Cron RQ. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. Genes Immun 2012;13:289-298.

6. Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. Curr Opin Rheumatol 2010;22:561-566

7. Ravelli A. Macrophage activation syndrome. Curr Opin Rheumatol 2002;14:548-552.

8. Behrens EM, Beukelman T, Paessler M, Cron RQ. Occult macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis. J Rheumatol 2007;34:1133-8.

9. Bleesing J, Prada A, Siegel DM, Villanueva J, Olson J, Ilowite NT, et al. The diagnostic significance of soluble CD163 and soluble interleukin-2 receptor alpha chain in macrophage activation syndrome and untreated newonset systemic juvenile idiopathic arthritits. Arthritis Rheum 2007;56:965-971.

10. Minoia F, Davì S, Horne A, Demirkaya E, Bovis F, Caifeng L, et al. Clinical features, treatment and outcome of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis A multinational, multicenter study of 362 patients. Arthritis Rheumatol 2014;66:3160-9

11. Minoia F, Davì S, Horne A, Bovis F, Demirkaya E, Akikusa J, et al. Dissecting the heterogeneity of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. J Rheumatol 2015 (In press).

12. Bode SF, Lehmberg K, Maul-Pavicic A, Vraetz T, Janka G, Stadt UZ, et al. Recent advances in the diagnosis and treatment of hemophagocytic lymphohistiocytosis. Arthitis Res Ther 2012;14: 213.

13. Aricò M, Janka G, Fischer A, Henter JI, Blanche S, Elinder G, et al. FHL Study Group of the Histiocyte Society. Hemophagocytic lymphohistiocytosis: report of 122 children from the International Registry. Leukemia 1996;10:197–203.

14. Ho C, Yao X, Tian L, Li FY, Podoltsev N, Xu ML. Marrow assessment for hemophagocytic lymphohistiocytosis demonstrates poor correlation with disease probability. Am J Clin Pathol 2014;141:62–71.

15. Henter JI, Horne A, Aricò M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.

16. Ravelli A, Magni-Manzoni S, Pistorio A, Besana C, Foti T, Ruperto N, et al. Preliminary diagnostic guidelines for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. J Pediatr 2005;146:598-604.

17. Davì S, Minoia F, Pistorio A, Horne A, Consolaro A, Rosina S, et al. Performance of current guidelines for diagnosis of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. Arthritis Rheumatol 2014;66:2871-80.

18. Weaver LK, Behrens EM. Hyperinflammation, rather than hemophagocytosis, is the common link between macrophage activation syndrome and hemophagocytic lymphohistiocytosis. Curr Opin Rheumatol 2014;26:562-569.

19. Zhang M, Behrens EM, Atkinson TP, Shakoory B, Grom AA, Cron RQ. Genetic defects in cytolysis in macrophage activation syndrome. Curr Rheumatol Rep. 2014;16:439.

20. Kaufman KM, Linghu B, Szustakowski JD, Husami A, Yang F, Zhang K, et al. Whole exome sequencing reveals overlap between macrophage activation syndrome in systemic juvenile idiopathic arthritis and familial hemophagocytic lymphohistiocytosis. Arthritis Rheumatol 2014;66:3486-95.

21. Davì S, Consolaro A, Guseinova D, Pistorio A, Ruperto N, Martini A, et al. An international consensus survey of diagnostic criteria for macrophage activation syndrome in systemic juvenile idiopathic arthritis. J Rheumatol 2011;38:764-768.

22. Kelly A, Ramanan AV. Recognition and management of macrophage activation syndrome in juvenile arthritis. Curr Opin Rheumatol 2007;19:477-81.

23. Stephan J, Kone-Paut I, Galambrun C, Mouy R, Bader-Meunier B, Prieur A. Reactive haemophagocytic syndrome in children with inflammatory disorders: a retrospective study of 24 patients. Rheumatology 2001;40:1285–92.

24. Pelkonen P, Swanljung K, Siimes MA. Ferritinemia as an indicator of systemic disease activity in children with systemic juvenile rheumatoid arthritis. Acta Paediatr Scand 1986;75:64-68.

25. De Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio GR, Martini A. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 1991;34:1158-63

26. Nigrovic PA, Mannion M, Prince FH, Zeft A, Rabinovich CE, van Rossum MA, et al. Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. Arthritis Rheum 2011;63:545-55.

27. Ruperto N, Brunner HI, Quartier P, Constantin T, Wulffraat N, Horneff G, et al. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. N Eng J Med 2012;367:2396-406.

28. De Benedetti F, Brunner HI, Ruperto N, Kenwright A, Wright S, Calvo I, et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. N Eng J Med 2012;367:2385-95.

29. Strippoli R, Caiello I, De Benedetti F. Reaching the threshold: a multilayer pathogenesis of macrophage activation syndrome. J Rheumatol 2013;40:761-7.

30. Shimizu M, Nakagishi Y, Kasai K, Yamasaki Y, Miyoshi M, Takei S, et al. Tocilizumab masks the clinical symptoms of systemic juvenile idiopathic arthritis-associated macrophage activation syndrome: the diagnostic significance of interleukin-18 and interleukin-6. Cytokine 2012;58:287-94.

31. De Benedetti F, Scheider R, Weitzman S, Devlin C, Daimaru K, Yokota S, et al. Macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis treated with tocilizumab [Abstract]. Proceedings of the 30th Annual Meeting of the Histiocyte Society (Toronto, ON, 28-30 October 2014); page 56.

32. Grom AA, Brunner HI, Ruperto N, Martini A, Lovell D, Pascual V, et al. Canakinumab in Systemic Juvenile Idiopathic Arthritis: Impact on the Rate and Clinical Presentation of Macrophage Activation Syndrome. Ann Rheum Dis 2014;73(Suppl. 2):578 (Abstract).

ACKNOWLEDGEMENTS

The authors thank the PRINTO employees Simona Angioloni, Chiara Pallotti, Michele Pesce, Mariangela Rinaldi and Luca Villa for the technical assistance and for providing the technical and secretarial assistance in the organization of the consensus conference. The authors thank the following physicians who contributed in the study by including their patients' data: Mario Abinun, MD (Newcastle, UK); Amita Aggarwal, MD (Lucknow, India); Jonathan Akikusa, MD (Melbourne, Australia); Sulaiman M. Al-Mayouf, MD (Riyadh, Saudi Arabia); Maria Alessio, MD (Napoli, Italy); Jordi Anton, MD (Barcelona, Spain); Maria Teresa Apaz, MD (Cordoba, Argentina); Itziar Astigarraga, MD (Bilbao, Spain); Nuray A. Ayaz, MD (Istanbul, Turkey); Patrizia Barone, MD (Catania, Italy); Blanca Bica, MD (Rio de Janeiro, Brazil); Isabel Bolt, MD (Berne, Switzerland); Luciana Breda, MD (Chieti, Italy); Vyacheslav Chasnyk, MD (Saint-Petersburg, Russian Federation); Rolando Cimaz, MD (Firenze, Italy); Fabrizia Corona, MD (Milan, Italy); Ruben Cuttica, MD (Buenos Aires, Argentina); Gianfranco D'Angelo, MD (Ancona, Italy); Zane Davidsone, MD (Riga, Latvia); Carmen De Cunto, MD (Buenos Aires, Argentina); Jaime De Inocencio, MD (Madrid, Spain); Eli Eisenstein, MD (Jerusalem, Israel); Sandra Enciso, MD (Mexico City, Mexico); Graciela Espada, MD (Buenos Aires, Argentina); Michel Fischbach, MD (Muenster, Germany); Michael Frosch, MD (Muenster, Germany); Romina Gallizzi, MD (Messina, Italy); Maria Luz Gamir, MD (Madrid, Spain); Yi-Jin Gao, MD (Shangai, China); Thomas Griffin, MD (Charlotte, USA); Soad Hashad, MD (Tripoli, Lybia); Teresa Hennon, MD (Buffalo, USA); Gerd Horneff, MD (Sankt Augustin, Germany); Zeng Huasong, MD (Guangzhou, China); Adam Huber, MD (Halifax, Canada); Norman Ilowite, MD (New York, USA); Antonella Insalaco, MD (Roma, Italy); Maka Ioseliani, MD (Tbilisi, Georgia); Marijia Jelusic-Drazic, MD (Zagreb, Croatia); Michael Jeng, MD (Stanford, USA); Agneza Kapovic, MD (Zagreb, Croatia); Ozgur Kasapcopur, MD (Istanbul, Turkey); Toshiyuki Kitoh, MD (Nagakute, Japan); Isabelle Kone-Paut, MD (Paris, France); Sheila

Knupp Feitosa de Oliveira, MD (Rio de Janeiro, Brazil); Bianca Lattanzi, MD (Ancona, Italy); Loredana Lepore, MD (Trieste, Italy); Caifeng Li, MD (Beijing, China); Jeffrey M. Lipton, MD (New York, USA); Silvia Magni-Manzoni, MD (Rome, Italy); Despoina Maritsi, MD (Athens, Greece); Deborah McCurdy, MD (Orange, USA); Rosa Merino, MD (Madrid, Spain); Velma Mulaosmanovic, MD (Sarajevo, Bosnia and Herzegovina); Susan Nielsen, MD (Copenhagen, Denmark); Priyankar Pal, MD (Kolkata, India); Sampath Prahalad, MD (Atlanta, USA); Donato Rigante, MD (Roma, Italy); Ingrida Rumba-Rozenfelde, MD (Riga, Latvia); Claudia Saad Magalhaes, MD (Botucatu, Brazil); Helga Sanner, MD (Oslo, Norway); Sujata Sawhney, MD (New Delhi, India); Wafaa M. Sewairi, MD (Riyadh, Saudi Arabia); Bita Shakoory, MD (Philadelphia, USA); Susan Shenoi, MD (Seattle, USA); Artur Silva Clovis, MD (Sao Paulo, Brazil); Valda Stanevicha, MD (Riga, Latvia); Kimo C. Stine, MD (Little Rock, USA); Gordana Susic, MD (Belgrade, Serbia); Flavio Sztajnbok, MD (Rio de Janeiro, Brazil); Syuji Takei, MD (Kagoshima City, Japan); Hasan Tezer, MD (Ankara, Turkey); Ralf Trauzeddel, MD (Berlin, Germany); Elena Tsitsami, MD (Athens, Greece); Erbil Unsal, MD (Izmir, Turkey); Olga Vougiouka, MD (Athens, Greece); Lehn K. Weaver, MD (Philadelphia, USA); Jennifer Weiss, MD (Hackensack, USA); Sheila Weitzman, MD (Toronto, Canada); Mabruka Zletni, MD (Tripoli, Lybia).

Table 1. Comparison of clinical and laboratory features at disease onset between patients classified as MAS or non-MAS by the expert panel.

		Patients with MAS		Patients with no MAS	
	N	(n = 95)	N	(n = 296)	*P*
Clinical manifestations[£]					
Fever	94	93 (98.9)	294	278 (94.6)	0.08
Hepatomegaly	94	68 (72.3)	295	75 (25.4)	<.0001
Splenomegaly	92	53 (57.6)	294	67 (22.8)	<.0001
Lymphadenopathy	91	48 (52.8)	292	73 (25.0)	<.0001
Active arthritis	95	63 (66.3)	295	163 (55.3)	0.06
Central nervous system involvement	93	40 (43.0)	292	25 (8.6)	<.0001
Hemorrhagic manifestations	92	25 (27.2)	294	16 (5.4)	<.0001
Heart involvement	94	27 (28.7)	294	34 (11.6)	<.0001
Lung involvement	95	27 (28.4)	294	40 (13.6)	0.0009
Kidney involvement	95	16 (16.8)	295	16 (5.4)	0.0004
Laboratory tests[§]					
Hemoglobin, g/dl	95	9.9 (8.0 – 11.2)	289	10.9 (9.4 – 12.2)	<.0001
White blood cell count, x 10^9/liter	95	8.1 (3.2 – 12.8)	289	15.3 (9.9 – 20.1)	<.0001
Neutrophil count, x 10^9/ liter	82	3.7 (1.5 – 8.0)	236	9.4 (5.1 – 14.2)	<.0001
Platelet count, x 10^9/ liter	95	98 (57 – 141)	290	385 (286 – 551)	<.0001
ESR, mm/hour	90	28 (17 – 65)	245	70 (39 – 93)	<.0001

C-reactive protein, mg/dl	85	8.7 (2.4 – 16.1)	282	8.2 (2.4 – 15.6)	0.63
Aspartate aminotransferase, units/ liter	93	171 (98 – 436)	284	30 (22 – 45)	<.0001
Alanine aminotransferase, units/liter	91	115 (43 - 283)	284	18 (12 - 34)	<.0001
Lactate dehydrogenase, U/liter	81	1,560 (801 – 2,400)	248	482 (362 – 688)	<.0001
Triglycerides, mg/dl	86	267 (192 – 358)	186	123 (96 – 160)	<.0001
Albumin, gm/dl	79	3.0 (2.6 – 3.5)	252	3.7 (3.2 – 4.1)	<.0001
Serum sodium, mEq/liter	80	136 (133 - 140)	259	138 (136 - 141)	0.003
Fibrinogen, mg/dl	88	220 (148 – 345)	226	500 (356 – 650)	<.0001
Ferritin, ng/ml	90	9,094 (2,000 – 19,767)	244	268 (62 – 938)	<.0001
D-dimer, ng/ml	48	3,579 (1,834 – 7,373)	94	1,638 (528 – 3,325)	<.0001

[£]Data are the number (percentage). [§]Values are the median (interquartile range)

MAS = macrophage activation syndrome; ESR = erythrocyte sedimentation rate

Table 2. Univariate analysis of the ability of variables to discriminate patients with MAS from comparison patients (n = 391).

	n	OR	95% CI	*P*
Clinical features				
Central nervous system involvement	385	8.1	4.5 – 14.4	<.0001
Hepatomegaly	389	7.7	4.5 – 12.9	<.0001
Hemorrhagic manifestations	386	6.5	3.3 – 12.8	<.0001
Fever	388	5.4	0.7 – 40.9	0.106
Splenomegaly	386	4.6	2.8 – 7.6	<.0001
Lymphoadenopathy	383	3.4	2.1 – 5.5	<.0001
Kidney involvement	390	3.5	1.7 – 7.4	0.0008
Heart involvement	388	3.1	1.7 – 5.5	0.0001
Lung involvement	389	2.5	1.4 – 4.4	0.0011
Active arthritis	390	1.6	1.0 – 2.6	0.0587
Laboratory features (non-normalized values)				
Ferritin > 684, ng/ml	334	111.6	26.7 – 465.8	<.0001
Platelet count ≤ 181 ,x 10^9/liter	385	84.3	40 – 177.5	<.0001
Aspartate aminotransferase > 48, units/liter	377	51.9	21.7 – 124.4	<.0001
Lactate dehydrogenase > 853, U/liter	329	20.0	10.7 – 37.3	<.0001
Triglycerides > 156, mg/dl	272	19.6	9.4 – 40.8	<.0001
Alanine aminotransferase > 36, units/liter	375	18.0	9.4 – 34.3	<. 0001

Fibrinogen ≤ 360, mg/dl	314	11.5	6.3 – 21.0	<.0001
Neutrophil count ≤ 3.7, x 10^9/liter	318	5.9	3.4 – 10.5	<.0001
D-dimer > 1350, ng/ml	142	5.7	2.4 – 13.4	<.0001
ESR ≤ 30, mm/hour	335	5.6	3.3 – 9.5	<.0001
Albumin ≤ 3.6, gm/dl	331	5.5	3.0 – 10.1	<.0001
Hemoglobin ≤ 8.5, gm/dl	384	4.9	2.7 – 8.8	<.0001
White blood cell count ≤ 10.2, x 10^9/liter	384	4.6	2.8 – 7.5	<.0001
Serum sodium ≤ 133, mEq/liter	339	2.9	1.7 – 5.0	0.0002
C-reactive protein > 0.86	367	2.4	1.0 – 5.9	0.0501
Laboratory features (normalized values)				
Ferritin > 2773.6, ng/ml	334	23.9	12.7 – 44.8	<.0001
Aspartate aminotransferase > 44.4, units/liter	377	47.4	21.6 – 103.9	<.0001
Alanine aminotransferase > 23, units/liter	375	39.4	14.1 – 110.5	<.0001
Lactate dehydrogenase > 238.8, U/liter	329	39.2	18.2 – 84.6	<.0001
Triglycerides > 193.9, mg/dl	272	16.3	8.7 – 30.8	<.0001
Fibrinogen ≤ 318.9, mg/dl	314	11.0	6.1 – 20.0	<.0001
Albumin ≤ 3.9, gm/dl	331	6.4	3.6 – 11.3	<.0001
D-dimer > 1320.3, ng/ml	142	5.2	2.1 – 12.7	<.0001
Serum sodium ≤ 135, mEq/liter	339	3.3	1.9 – 5.6	<.0001

OR = odds ratio; 95% CI = 95% confidence interval.

Table 3. The classification criteria for macrophage activation syndrome in systemic juvenile idiopathic arthritis

A febrile patient with known or suspected systemic juvenile idiopathic arthritis is classified as having macrophage activation syndrome if the following criteria are met:
Ferritin > 684 ng/ml
and
any 2 of the following:
Platelet count ≤ 181 x10^9/liter
Aspartate aminotransferase > 48 U/liter
Triglycerides > 156 mg/dl
Fibrinogen ≤ 360 mg/dl

Laboratory abnormalities should not be otherwise explained by the patient's condition, such as concomitant immune-mediated thrombocytopenia, infectious hepatitis, visceral leishmaniasis, or familial hyperlipidemia.

Table 4. Logistic regression model to assess the association of the variables included in the final classification criteria and the web-based experts' consensus of patients' classification as MAS or non-MAS*

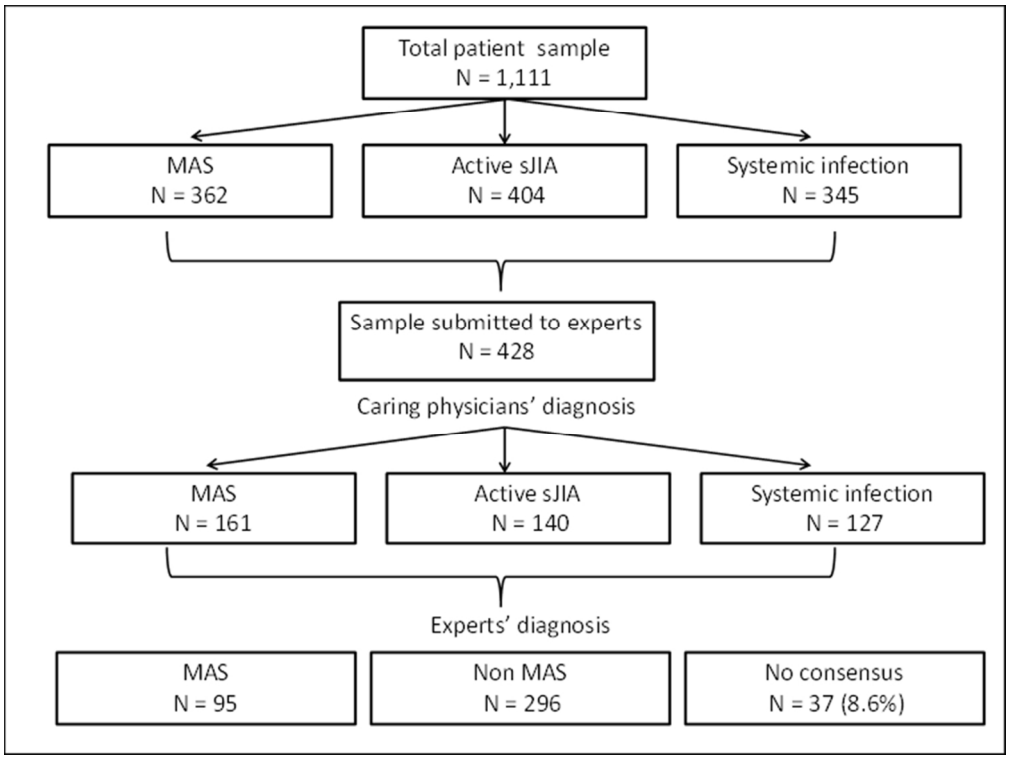
Explanatory variable	OR	95% CI	$P^{\S}$
Ferritin > 684 ng/ml	>999.999	40.6 - >999.999	<.0001
Platelet count ≤ 181 x10^9/l	237.0	18.2 - >999.999	<.0001
Triglycerides > 156 mg/dl	18.3	2.0 – 163.9	0.009
Fibrinogen ≤ 360 mg/dl	16.0	2.0 – 126.4	0.009
Aspartate aminotransferase > 48 U/l	10.0	1.3 – 78.4	0.029

*The web-based experts' consensus on patients' classification as MAS or non-MAS was the dependent variable. Complete data were available on 227 patients. The area under the receiver operating characteristic curve of the model was 0.99. OR = odds ratio; 95% CI = 95% confidence interval.

§By likelihood ratio test

LEGEND TO FIGURE

Figure 1. Patient samples evaluated in the study and results of web-based expert evaluations



Patient samples evaluated in the study and results of web-based expert evaluations
127x95mm (300 x 300 DPI)

Online supplementary Table T1. Composition and statistical performance of the top 20 classification criteria

N	Classification criteria	Derivation	Sensitivity	Specificity	PPV	NPV	AUC	kapp
929	Ferritin >684 ng/ml (84); PLT ≤ 181 x10^9/liter (13); AST > 48 U/l (1); Triglycerides >156 mg/dl (1); Fibrinogen < 360 mg/dl (1) - Total score > 86	MS	0.95	1.0	100	97.4	0.97	0.96
932	Ferritin >684 ng/ml (84); PLT ≤ 181 x10^9/ liter (13.5); Triglycerides >156 mg/dl (1.5); Fibrinogen < 360 mg/dl (1) - Total score > 85.5	MS	0.95	0.99	98.7	97.4	0.97	0.95
943	Ferritin >684 ng/ml (70); PLT ≤ 181 x10^9/ liter (25); Triglycerides >156 mg/dl (2); Fibrinogen < 360 mg/dl (3) - Total score > 73	MS	0.95	0.99	98.7	97.4	0.97	0.95
941	Ferritin >684 ng/ml (68); PLT ≤ 181 x10^9/ liter (25); Triglycerides >156 mg/dl (1); Fibrinogen < 360 mg/dl (3); Albumin ≤ 3.6 g/dl (3) - Total score > 72	MS	0.95	0.99	96.9	97.9	0.97	0.94
976	Ferritin >684 ng/ml (59); PLT ≤ 181 x10^9/ liter (39); Fibrinogen < 360 mg/dl (1); Hepatomegaly (1) - Total score > 60	MS	0.95	0.99	96.4	98.1	0.97	0.94
930	Ferritin >684 ng/ml (84.5); PLT ≤ 181 x10^9/ liter (13); AST > 48 U/l (1); Triglycerides >156 mg/dl (1.5) - Total score > 86	MS	0.96	0.97	93.9	98.1	0.97	0.93
931	Ferritin >684 ng/ml (84.5); PLT ≤ 181 x10^9/ liter (13.5); AST > 48 U/l (1); Fibrinogen < 360 mg/dl (1) - Total score > 85.5	MS	0.95	0.98	95.2	98.1	0.97	0.93
942	Ferritin >684 ng/ml (68); PLT ≤ 181 x10^9/ liter (25); Fibrinogen < 360 mg/dl (3); Albumin ≤ 3.6 g/dl (3) - Total score > 72	MS	0.93	0.99	97.1	97.4	0.96	0.93
529	At least 4 of the following: Ferritin > 2774 ng/ml; PLT < 181 x10^9/ liter;	CC	0.93	0.98	95.4	97.7	0.96	0.92

	AST > 44.4 U/l; Triglycerides > 194 mg/dl; Fibrinogen < 319 mg/dl; Hepatomegaly							
798	Ferritin ≥ 500 ng/ml + any 2 of the following: PLT ≤ 262 x10^9/ liter; AST > 59 U/l; WBC ≤ 4 x10^9/l; Fibrinogen ≤ 250 mg/dl	CC	0.94	0.98	94.4	97.9	0.96	0.92
934	Ferritin >684 ng/ml (85.5); PLT ≤ 181 x10^9/ liter (13.5); Fibrinogen < 360 mg/dl (1) - Total score > 85.5	MS	0.95	0.97	92.1	98.0	0.96	0.91
935	Ferritin >684 ng/ml (85); PLT ≤ 181 x10^9/ liter (13.5); Triglycerides >156 mg/dl (1.5) - Total score > 86.5	MS	0.88	1.0	100	94.2	0.94	0.91
946	Ferritin >684 ng/ml (71); PLT ≤ 181 x10^9/ liter (26); Fibrinogen < 360 mg/dl (3) - Total score > 71	MS	0.95	0.97	92.1	98.0	0.96	0.91
947	Ferritin >684 ng/ml (72); PLT ≤ 181 x10^9/ liter (26); Triglycerides >156 mg/dl (2) - Total score > 74	MS	0.88	1.0	100	94.2	0.94	0.91
977	Ferritin >684 ng/ml (60); PLT ≤ 181 x10^9/ liter (39); Fibrinogen < 360 mg/dl (1) - Total score > 60	MS	0.95	0.97	92.1	98.0	0.96	0.91
307	Ferritin > 684 ng/ml and PLT ≤ 181 x10^9/ liter	CC	0.86	1.0	100	94.9	0.93	0.90
466	Ferritin > 684 ng/ml and PLT ≤ 181 x10^9/ liter + any 1 of the following: AST > 48 U/l; Triglycerides > 156 mg/dl; Fibrinogen < 360 mg/dl	CC	1.0	0.95	85.6	100	0.97	0.90
472	Ferritin > 684 ng/ml + any 2 of the following: PLT ≤ 181 x10^9/ liter; AST > 48 U/l; Triglycerides > 156 mg/dl; Fibrinogen ≤ 360 mg/dl	CC	0.97	0.96	89.7	98.7	0.96	0.90
481	Any 3 of the following: Ferritin > 2774 ng/ml; PLT < 181 x10^9/ liter; AST > 44.4 U/l; Triglycerides > 194 mg/dl; Fibrinogen < 319 mg/dl)	COC	0.96	0.96	89.2	98.6	0.96	0.90
580	Ferritin > 684 ng/ml and PLT < 181 x10^9/ liter + any 1 of the following:	CC	0.86	1.0	100	94.9	0.93	0.90

	AST > 48 U/l; Triglycerides > 156 mg/dl; Fibrinogen < 360 mg/dl; Hepatomegaly

PLT = platelet count; AST = aspartate aminotransferase; MS = criteria derived from the MAS score method (see text for explanation); CC = criteria derived through the combination of criteria approach (see text for explanation); PPV = positive predictive value; NPV = negative predictive value; AUC: area under the curve. The number in parenthesis for the items included in the criteria derived through the MS method are the weights yielded by multivariable logistic regression analyses.